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Research Paper

Glyphosate Contamination at Several Concentrations And Its Impact on Changes in Goat (Capra hircus) Organ Tissue

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Abstract
Herbicides are generally used in the control of weeds in plants. The use of synthetic chemicals such as herbicides is known to have a negative impact on the environment. This is because the chemicals in the herbicide can kill or poison the organisms that eat plants that are exposed to these chemicals. Glyphosate is one of the active ingredients of herbicides which is systemic, so it may leave a residue on weeds such as grasses which are usually used as animal feed. The aim of this study is to analyze the impact of glyphosate residues on changes in the organ tissue of goats (*Capra hircus*) fed with feed exposed to glyphosate compounds. The study was conducted using an experimental method with four treatments of glyphosate concentration including control. The treatment consists of four goats with age of 12 months. The results showed that glyphosate treatment in feed influenced the development and tissue of the goats. The higher the concentration of the residue exposed to the feed, the higher the residual concentration of glyphosate in livestock blood, namely 0.2377 ppm (P1), 0.3118 ppm (P2), and 0.9377 ppm (P3), respectively. The results of the observation on livestock organ tissue showed that there was severe damage to the liver and minor damage to the lung, gastric and kidney tissue. The higher the concentration of the residue exposed to the feed, the higher the residual concentration of glyphosate in livestock blood and causing more damage.

Keywords
Glyphosate compounds, animals feed, goat organ tissue

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1. INTRODUCTION

Herbicides are commonly used in both small-scale and large-scale agriculture and pose a risk of residue on grasses commonly used by farmers for animal feed (Vicini et al., 2019; Sørensen et al., 2020). Glyphosate is one of the active ingredients of various non-selective herbicides which is quite dangerous because it can inhibit the synthase of 5-enolpyruvylshikimate 3-phosphate (EPSP), the shikimate pathway enzyme present in plant cells (Vicini et al., 2019).

These compounds have a large number of tumorigenic effects on biological systems, including direct damage to cell DNA, disruption of glycine homeostasis, inhibition of succinate dehydrogenase, modification to more carcinogenic molecules such as N-nitrosoglyphosate and glyoxylates as well as disturbances in fructose metabolism which have the potential to cause adverse effects on animal reproduction. in the form of damage to reproductive tissue and damage to gametogenesis (Samsel and Seneff, 2013; Jarrell et al., 2020).

According to Torretta et al. (2018), exposure to glyphosate can be through the digestive tract of mammals, inhalation, food consumption and skin contact. Glyphosate was given orally in high doses to mice, causing growth retardation, kidney damage, liver enlargement, inflammation and gastric disease. Glyphosate is absorbed and distributed through the blood, in mammals <1% is metabolized to aminomethylphosphonic acid, then rapidly absorbed and excreted in the urine as parent compounds (ATSDR, 2020). Systemic intoxication in animals is characterized by gastrointestinal and neurological, mucosal irritation of the kidneys and gastrointestinal tract. Glyphosate exposure at low doses can cause endocrine system disorders, hepatorenal damage, birth defects, teratogenic effects and changes in the microbiome in mammals and insects (, EFSA; Beecraft and Rooney, 2021).

Animals exposed to the compound glyphosate can develop ataxia, difficulty in breathing and occasionally have

seizures and die. This compound has acute toxicity on oral, dermal and inhalation exposure. Eye or mucosal irritation occurs when these compounds are in target organs, namely the intestinal tract, salivary glands, liver and bladder (Anadón et al., 2009; EFSA, 2016).

This study aims to determine the effect of glyphosate compounds on blood plasma and organ tissue damage of goats exposed to the herbicide glyphosate.

2. EXPERIMENTAL SECTION

2.1 Study area

The study was conducted in the livestock area of the Animal Husbandry Departement, Faculty of Agriculture, Sriwijaya University, Indralaya Ogan Ilir Campus, South Sumatra Indonesia (3°13'18.1"S 104°38'48.7"E) and Anatomical Pathology Diagnosis Center Palembang (2°58'00.9"S 104°44'41.7"E).

Research Ethics

This study was accepted by the ethics commission, then this study was approved and declared ethically worthy with a certificate of approval number: 108/UN9.1.10/KKE/2020

2.2 Methods

The study was conducted in the livestock area, Animal Husbandry Departement, Faculty of Agriculture, Sriwijaya University Ogan Ilir and the Laboratory of Anatomical Pathology Diagnosis Center, Palembang, South Sumatra Indonesia, from June 2020 to January 2021. The study used an experimental method with 4 treatment groups, consisting of control (P0), Goats that were given feed exposed to 0.50 g/kgDM glyphosate (P1), goats that were given feed exposed to 1.001 g/kgDM glyphosate (P2), and goats that were given feed exposed to 1.50 g/kgDM glyphosate (P3). The treatmeant consists of 4 goats sample with age of 12 months and an average body weight of 29 kg.

The goats were adapted for 3 days prior to the treatment of forage feeding in the form of green grass which was commonly consumed according to the treatment and drank water ad libitum. Feed is given in the morning and evening, each as much as 3 kg/goat. The feed used in this study is the forage grass which is commonly used by breeders. Then the forage is cleaned and spread out on a tarpaulin and then treated with glyphosate according to the treatment and left for 10 minutes before giving it to the goats. Glyphosate-exposed feed was given for 45 days. The parameters observed were goat behavior, glyphosate concentration in blood and organ tissue histopathology including muscle, rumen, lungs, kidney, and liver.

Check the concentration of glyphosate in the blood. Blood is drawn from the jugular vein by venoject and collected in a blood plasma tube. All samples were stored at 18°C for a maximum of 7 days prior to testing. Measurement of glyphosate concentration levels using the Liquid chromatography-mass spectrometry (LCMS) method.

Tissue Histopathology Analysis. The procedure for making histopathological preparations, namely using microanatomy preparations of meat, rumen organ tissue, lungs and kidneys, was made using the paraffin method and haematoxin-eosin (HE) staining. The preparation output was then documented and observed visually microscopically. Furthermore, microscopic analysis was carried out on the organ tissues of the flesh, stomach, kidneys, lungs, and liver to analyze the effect of exposure to glyphosate compounds (Heymann et al., 2021).

3. RESULTS AND DISCUSSION

3.1 Behavior of goats after being treated feed (forage) thas has been exposed to glyphosate.

The results of the observation of goats (*Capra hircus*) that were fed with feed exposed to glyphosate showed differences with the control group. The goats fed with glyphosate-exposed forage are seen sticking out their tongues, blowing air through their mouths, consuming lots of drinking water, several times spitting out their tongues as if they are tasting unusual taste. Meanwhile, goats in the control group behave normally.

3.2 Glyphosate concentration in goat blood plasma

The results of the analysis of glyphosate concentrations in the blood showed that the higher the glyphosate concentration exposed to the feed, the higher the glyphosate concentration in goat blood (Table 1).

Table 1. Glyphosate residual concentrations in goat blood plasma

P0 (Control)	Glyphosate concentrarion (ppm)
P0 (Control)	0
P1 (0.50 g/kgDM)	0.2377
P2 (1.00 g/kgDM)	0.3118
P3 (1.50 g/kgDM)	0.9377

Research results on blood plasma, from various doses of glyphosate, namely P1 (0.50 g/kgDM) to P3 (1.50 g/kgDM) given to forage feed, caused an increase in glyphosate concentration along with the increase in the treatment dose in experimental animals. Blood plasma functions as a transportation medium to deliver nutrients to cells from various organs of the body and transports the rest of the cell metabolism to the kidneys, liver, and lungs to be secreted (Yawn, 2020). Leukocytes are part of the immune system that participate in the immune response (Malech, 2014).

Giving glyphosate orally with daily doses of 50, 100 and 150 mg/bb, given for 15, 30. and 45 days, resulted in an increase in the number of leukocytes associated with the given dose and a decrease in erythrocytes and hemoglobin (Naz et al., 2019). Glyphosate is an organophosphate that inhibits the activity of the cytochrome P450 enzyme and

is an overlooked component of toxicity for mammals. CYP enzymes play an important role, one of which is xenobiotic detoxification. The negative impact of glyphosate in the body is very dangerous, namely the occurrence of inflammation that damages cells in systems throughout the body (Samsel and Seneff, 2015). Glyphosate at high concentrations from 0.5 to 10 mM can cause DNA damage (Kwiatkowska et al., 2017). From the results of the analysis, it can be understood the fact that the higher the glyphosate concentration given to forage, the higher the glyphosate concentration in blood plasma of goats *(Capra hircus)*.

3.3 Histopatology of organ tissue

The results of histopathological analysis of the tissue of the rumen, lungs, kidneys and liver of goats *(Capra hircus)*, which consumed feed exposed to glyphosate, showed gradual changes in histological abnormalities that were getting worse with increasing treatment, especially in the rumen, lungs, kidneys and liver (Table 2). The glyphosate exposure in treatment P1 and P2 did not affect the development of goat meat tissue, as shown from the results of hispatology, all treatments were not different from the control treatment. Treatment P3 showed the occurrence of mild inflammation of the muscle fiber tissue (Figure 1).

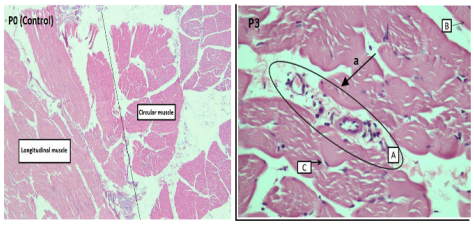

Figure 1. Effect of feed exposed to glyphosate on the development of muscle tissue/goat meat histopathology (P0, normal tissue; P3, muscle tissue/goat meat histopathology in P3 treatment, the occurrence of inflammation (A, B and C) is seen in the peripheral tissue of muscle fibers)

The results showed that the various doses of glyphosate that were given to the goat forage did not cause any significant changes in the histological structure of the muscles. There was no inflammation found in the P1 (0.50 g/kgDM) and P2 (1.001 g/kgDM) treatment groups. However, there was mild inflammation in the P3 treatment group (1.50 g/kgDM).

The epimysium is the epimysial layer that describes the superficial surface of the epimysium. The epimysium consists of collagen bundles in a crimp pattern. Epimysium stiffness increases with age and response to injury (Gillies and Lieber, 2011). Inflammation of the skeletal muscles begins with the activation of several signaling and anti-inflammatory pathways such as macrophages and neutrophils (Mukund and Subramaniam, 2020). A research conducted by Hardy et al. (2016) described the occurrence of myofibre necrosis

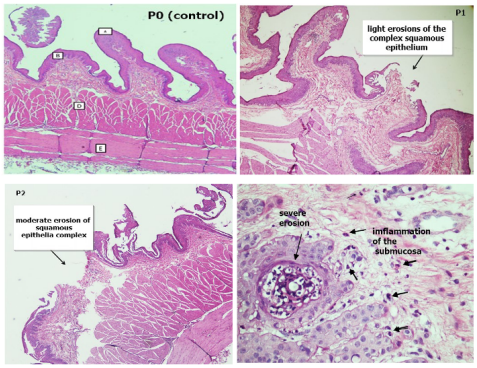

Figure 2. Effect of feed exposed to glyphosate compounds on the development of rumen tissue in goats. The occurrence of erosion is getting heavier with increasing exposure to glyphosate in feed

followed by recovery of fiber neosynthetic tissue.

Histopathological observations on muscle tissue showed that the herbicide glyphosate could cause damage to muscle tissue, which showed a mild inflammatory cell in the peripheral muscle fibers in the P3 treatment group. Thus, the mild inflammatory cells found in the goat muscle samples occurred due to glyphosate herbicide poisoning from glyphosate exposure to forage feed.

Microscopic observations of rumen tissue showed changes in development when compared to control group. In the P1 treatment group, it was seen that the rumen tissue had erosion on the mucosa. Erosion is getting heavier with increasing exposure to glyphosate compounds in the feed consumed by goats (P2 and P3). In P3 treatment, besides the increasingly severe erosion, inflammation of the submucosa was also found (Figure 2).

The observation on rumen tissue samples treated with glyphosate in feed showed that, giving various doses of glyphosate P1 (0.50 g/kgDM) to P3 (1.50 g/kgDM) caused erosive foci and increased erosion along with with increasing the dose of treatment in experimental animals.

Gastric erosion is a defect in the superficial mucosa and erosion often occurs in the gastric part of the antrum. Normally the gastric mucosa is protected by stomach acid (Robert, 2006). The rumen in mammals generally contains a lot of rumen microbiota which contribute to the fermentation process (Zhang et al., 2019). The rumen is lined with papillae for nutrient absorption and is divided by muscle pillars into dorsal, ventral, coudodorsal and coudoventral sacs. The rumen is a place for microbial fermentation, cellusose microorganisms from the cell walls, digesting complex starches, synthesizing protein from non-protein nitrogen and maintaining vitamin B and vitamin K. Rumen has a pH ranging from 6.5 to 6.8 (Parish et al., 2017).

The rumen epithelium has functions such as absorption, transportation, fatty acid metabolism and protection. The rumen epithelium is a stratified squamous epithelium consist-

Table 2. Effect of feed exposed to glyphosate on goat organ histopathology

	Glyphosate treatment of feed			
Organ/Tissue	Control	P1 (0.50 g/kgDM)	P2 (1.00 g/kgDM)	P3 (1.50 g/kgDM)
Muscle/flesh	Normal	Normal	Normal	The presence of mild inflammation of the peripheral muscle fibers
Gaster/rumen	Normal	There were erosive foci on the mucosa	Erosion more and greater than P1	Erosion more and greater than P2
Lung	Normal	Edematic alveoli and proliferation of pulmonary vessels	Edematic alveoli and proliferation of pulmonary vessels	Edematic alveoli and proliferation of pulmonary vessels
Kidney	Normal	The renal parenchyma with glomeruli and tubules is normal with designation of a mild the tubules	The renal parenchyma with glomeruli and tubules is normal with designation of a mild the tubules	The renal parenchyma with glomeruli and tubules is normal with a mild designation of the tubules The presence of infiltration in plasma lymphocytes and neutrophils
Liver	Normal	Cellular changes occur in the form of hydrophic degeneration, karyorexis, pycnosis and hydrophilic degeneration. There is a mild infiltration of lymphocytes, plasma and neutrophils	Cellular changes occur in the form of hydrophic degeneration, karyorexis and hapatic lobe pycnosis, moderate infiltration of lymphocytes, plasma cells and neutrophils.	Most of the tissue degeneration and necrosis is severe, filtration is quite heavy and there are gradual pathological changes.

ing of 4 parts, namely the stratum basale, stratum spinosum, stratum granulosum and stratum corneum (Baldwin and Connor, 2017). Research conducted by Krüger et al. (2013) stated that glyphosate can reduce lactic acid-producing bacteria such as lactobacillus, lactococci and enterococci in the digestive tract. Glyphosate can decrease beneficial bacteria in the microbiota of the digestive tract (Shehata et al., 2013). Research conducted by Ohfuji (2020), showed that rumen histopathology contained lesions, chronic erosive or ulcerative mucosal lesions as well as inflammatory lesions that showed eosinophil infiltrates in the rumen wall.

Results of histopathological observations on the rumen tissue showed that the herbicide glyphosate could cause damage to the rumen tissue which resulted in erosion. The erosion increased gradually with increasing glyphosate concentration of the treatment groups.

The effect of glyphosate on lung histopathology, indicating the occurrence of changes in tissue when compared to the control group. The histopathological picture of normal lungs, it appears that the bronchials are lined with ciliated columnar epithelium; there is cartilage tissue in the sub mucosa. The results of microscopic observations in the P2 and P3 treatment groups showed that there was a widening of the alveolar air cavity, the infection was mild to moderate consisting of lymphocytes and plasma cells. In the P3 treatment group, it was seen that the lung tissue in addition to experiencing widening of the air cavity, the infiltration also experienced hyaline thickening in the pulmonary blood vessels (Figure 3).

The results showed that various doses of glyphosate were given to goat forage, which caused a significant change in the histological structure of the lungs. Edematic alveoli and pulmonary blood vessel proliferation were found in the P1 (0.50 g/kgDM), P2 (1.001 g/kgDM) and P3 (1.50 g/kgDM) treatment groups.

The function of the lungs in mammals is gas exchange in the alveolar region (parenchyma). Air reaches the alveolar lumen through the airway conduction system and blood flows in the capillary network in the interalveolar septa (Knudsen and Ochs, 2018). In pulmonary edema, the alveolar is filled with fluid which causes mechanical pressure on the air-filled alveoli so that it is prone to injury due to excessive pressure

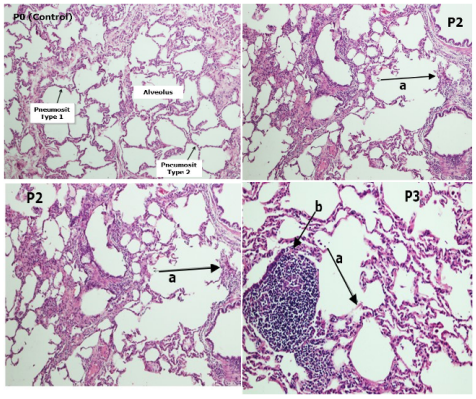

Figure 3. Effects of feed exposed to glyphosate compounds on the development of lung tissue in goats. Normal lung tissue (P0). Changes in lung tissue on treatment P1, P2 and P3 (a. Widening of the alveolar air cavity; b. Hyaline thickening of the pulmonary blood vessels)

during ventilation (Perlman et al., 2011). Poliferation causes hypertrophy of blood vessels and increases blood vessel pressure and increases in pulmonary artery blood pressure (Wilson et al., 2015).

Morphologically, the lungs are located in the thoracic cavity on both sides of the mediastinum and are covered by serous pleura. The pleura has two layers, namely parietal and visceral. Each lung is divided into the number of lobes then divided into lobules by interlobular tissue (Khyalia et al., 2019). The pulmonary alveoli in goats are coarse spherical structures that open into the alveolar ducts and alveolar sacs. The mean alveolar diameter was 45.28 ± 1.81 μm and the mean inter-alveolar thickness was 6.64 ± 0.35 μm (Baba and Choudhary, 2008). Lung abnormalities in goats vary in lobule and acini. Bronchi and bronchioles contain desquamated epithelial cells, alveolar macrophages, and neutrophils. In addition, there is thickening of the alveolar septa as a consequence of hyperplasia of connective tissue and smooth muscle cells (Panayotova-Pencheva and Alexandrov, 2010).

The histopathological observations on lung tissue indicate that the herbicide glyphosate can cause damage to lung tissue resulting in alveolar edema and the occurrence of pulmonary blood vessel proliferation. Thus the herbicide glyphosate has a toxic effect on livestock. Histopathological analysis on the organ tissue of goat (*Capra hircus*), in general, between control and treatment groups did not show a significant difference. However in P2 and P3 treatment groups, there were increase in the density of inflammatory cells in the tubules (Figure 4).

Based on the results of the study, it was shown that various doses of glyphosate that were given to goat feed caused a significant change in the histological structure of the kidneys. There were mild presence in the tubules in the P1 (0.50 g/kgDM) and P2 (1.001 g/kgDM) treatment groups.

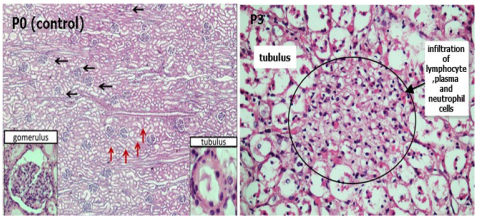

Figure 4. The effect of feed exposed to glyphosate compounds on the development of kidney tissue in goats

In the P3 treatment group (1.50 g/kgDM), in addition to being mild, there was infiltration in lymphocytes, plasma, and neutrophils.

Infiltration consists of the number of focus lymphocytes, plasma cells and magrophages in the tissue with little or no damage to the surrounding parenchyma, such as in the renal tubules (Patrick and Rebelatto, 2015). The renal system of the small ruminants includes the papillary ducts, renal crest, renal pelvis and ureters. The renal pelvis is the common cavity around the renal bounded by the epithelium where the renal papillae fuse and open, the tubules are lined by cuboidal epithelium. Papillary ducts are lined by cuboidal epithelium at the apex of the kidney or papilla (Venumadhav and Rajendranath, 2019).

The kidneys have a function as electrolyte homeostasis, removing toxic metabolic waste, especially those containing nitrogen compounds urea and creatinine (Jabbar et al., 2018). Serum creatinine is considered an important indicator of kidney health and is used for detection of damage to the nephrons (Karim and Abbas, 2021).

The observation of histopathological showed that there was a mild disease accompanied by infiltration of cells, neutrophil blood plasma lymphocytes in goat kidneys, thus the herbicide glyphosate had a toxic effect on livestock (Figure 5).

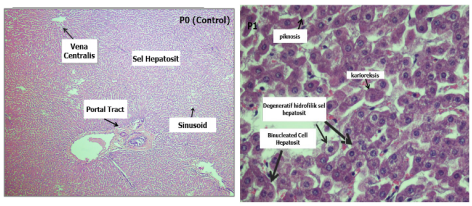

Figure 5. The effect of feed exposed to glyphosate compounds on the development of liver tissuein goats

Based on the research, the results obtained between the control group and all treatment groups have differences in macroscopic observations. In the macroscopic observation of goat liver in groups P1 to P2 there were changes in the form of hydrophic degeneration, karyorexis, pycnosis and hydrophilic degeneration. What distinguishes the P2 treatment group is there were occurrence of hepatic lobe

picnosis and moderate infiltration of lymphocytes, plasma cells and neutrophils. Whereas in P3 treatment group, some of the liver experienced severe degeneration and necrosis and the filtration was quite severe with gradual pathological changes.

Tissue necrosis is a process that results in irreparable damage to structure and function. Cell necrosis is usually a microscopic change in the nucleus. These changes include inflammation of the nucleus which is followed by nuclear chromatin condensation (pyknosis) and nucleus dissolution (caryolysis) (Bullough, 2010). Neutrophil infiltration plays a central role in inflammation and is also a major cause of tissue damage (Bian et al., 2012).

The liver is an important organ in xenobiotic detoxification (Peillex and Pelletier, 2020). Glyphosate administration in cattle feed with an average glyphosate intake of 122.7 μg/kg/ day for 16 days, affected the total blood blilirubin concentration levels (Heymann et al., 2021). The main function plays a role in determining the non-specific immune response of organisms and plays a role in adaptive or specific immune responses including T and B lymphocytes for defense from harmful pathogens (Jakab, 2015).

A study conducted by Saleh et al. (2018) shows that many changes occurred in the treatment group, where there was an increase in the alanine aminotransferase and aspartate aminotransferase enzymes, cellular infiltration, a lot of nuclear degeneration, physical necrosis, cytoplasm and lipid deposition, increase in the number of collagen fibers, and an increase in the number of mast cells.

The results of histopathological observations shows the occurrence of cell decline or cell abnormalities due to injury and premature death of cells and living tissue caused by exposure to glyphosate. The cells undergo hydropic degeneration where hydropic degeneration can result in the accumulation of fluid in cells, causing the liver volume to increase. Thus, the herbicide glyphosate has a toxic effect in livestocks.

4. CONCLUSIONS

Glyphosate treatment in feed influenced the development and tissue of the goats. The higher the concentration of the residue exposed to the feed, the higher the residual concentration of glyphosate in livestock blood and causing more damage.

5. Acknowledgement

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